“ESTIMATION OF PHYTOREMEDIAL CAPABILITY OF SOME ORNAMENTAL PLANTS AGAINST WATER AND SOIL POLLUTION IN SELECTED SITES OF DRAVYAVATI RIVER, JAIPUR, RAJASTHAN”

Research protocol

For Registration for Ph.D. Programme

Submitted By

Namrata Jadaun

Under the Guidance of:

Dr. Sonali Pandey

Professor, (Department of Botany)

JECRC University, Jaipur

2020

JECRC UNIVERSITY, JAIPUR

CONTENTS

Topics	Page No.
1. Introduction	3-10
2. Review of Literature	10-12
3. Statement of the Research Problem	13
4. Objectives of The Research work	13
5. Scope of the Study	13
6. Significance of the Study	14
7. Limitations	14
8. Methodology	14-16
9. Month Wise Plan of Work	17
10. Work Plan (Graphical Representation)	18
11. References	19-24

INTRODUCTION

The major environmental problem of today is soil and water pollution caused by heavy metals (Chhotu and Fulekar, 2009). Various types of pollution are produced and increases day by day due to many anthropogenic activities that are ultimately introduced into the Environment. A huge amount of chemical substances released in air, water, and soil through various processes harm the environment in various ways. Water and soil contaminated with heavy metals create many health problems related to humans and other animals. Heavy metals are among the most important source of contaminants (Tangahu *et al.*,2011). Heavy metals are very noxious which causes serious problems. Heavy metals that are mainly present in soil and water can be grouped in essential and non-essential, copper (Cu), nickel (Ni), manganese (Mn), iron (Fe) and zinc (Zn) are essential heavy metals which are required for persuing many biological and physiological activities of plants (Cempel and Nikel, 2006), but present in the excess amount they may become toxic. Non-essential heavy metal like lead (Pb), chromium (Cr), mercury (Hg), cadmium (Cd), and arsenic (As) are very toxic in less amount, not biologically degradable, may enter into the food chain by edible plants and accumulate in animals through biomagnification (Sarwar *et al.*,2010). The concentration of heavy metals varies between 1 to 100,000 mg/kg and they are classified as toxic (Blaylock and Huang, 2000). The presence of metals in excess amount in the soil results in decreased soil fertility, microbial activity and yields (McGrath *et al.*,1995). Heavy metals like copper (Cu), aluminium (Al), cadmium (Cd), lead (Pd) and chromium (Cr) could catalyze the production of Reactive Oxygen Species (ROS), this can cause damage to cytoplasmic molecules, DNA and proteins (Stanley *et al.*, 2018). Pesticides, insecticides, chemical fertilizers, kitchen waste, agricultural waste act as waste generation sources.

For remediation of heavy metals present in contaminated soil, there are many techniques used, such as acid leaching, excavation, landfill thermal treatment and electro reclamation but these are not suitable for practical application because of their low efficiency, large destruction of soil structure, soil property and their high cost. These problems lead to the development of phytoremediation as an effective and affordable strategy to remove heavy metals from contaminated soil (Chaney *et al.*, 2000).

Phytoremediation is the technology by which living plants are used to clean up contaminated water, soil, and air by detoxifying various compounds like non-degradable heavy metals, pesticides, explosives, and oil. Phytoremediation is the *in-situ* treatment of contaminated soil and water. Phytoremediation takes advantage of natural plant processes and requires less equipment and labour than the other techniques. It is a cost-effective method.

The Godfather of phytoremediation is Robert Richard Brooks of New Zealand. Phytoremediation is the word originated from the Greek prefix "Phyto" which means plants and suffix "remedium" meaning to clean (or) restore (Cunningham *et al*.,1996). United States patent was filled titled as "Phytoremediation of Metals" by a company called 'Phytotech' in 1993 (Patil and Gawande, 2018). The idea of using plants for removing metals and other compounds was introduced in 1983, but the concept has been executed from the last 300 years (Soni and Jain, 2014).

Ex-situ methods involve excavating the soil and burying it in another landfill site, however shifting the contamination problem elsewhere (Smith, 1993).
In-situ Methods perform remediation of contaminated soil at the same place and no need to excavation, so the *In-situ* process is always preferable.

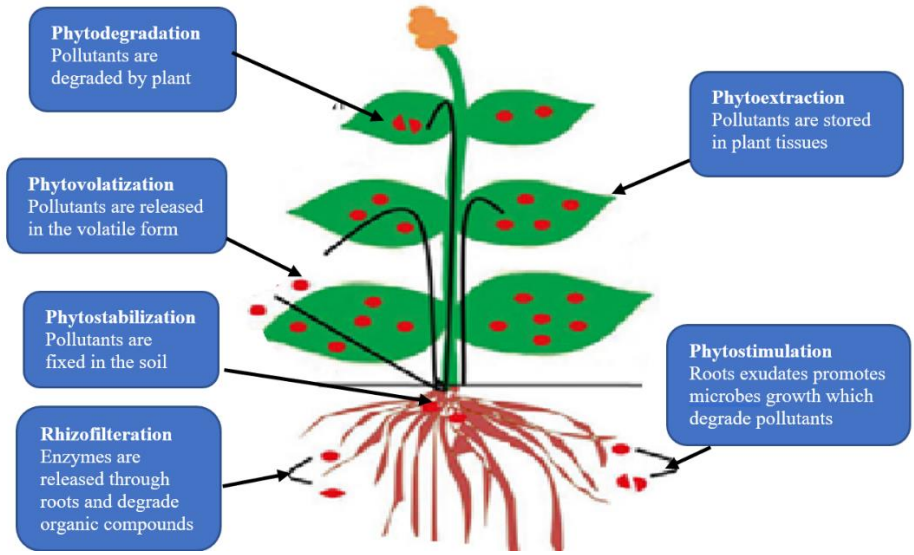
Plants are the universal producers of the ecosystem, humans and other animals are completely dependent directly or indirectly on plants, they provide oxygen directly for the survival of humans and other animals. They give us food, shelter, clothes and beautify our surroundings. Many of them are reported as metal accumulator like Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Fabaceae, Lamiaceae, Poaceae, Violaceae, Euphorbiaceae are some of the families associated with such plants (Prasad and Freitas, 2003). More than 721 plant species have been identified as metal hyper-accumulators (www.hyperaccumulators.org, 2015).
Brassicaceae family have the largest number of plant species as a metal accumulator. Nickel hyperaccumulation has been reported by almost 72 species of Brassicaceae family (Reeves *et al*., 1996).

Phytoremediation by flowering plants is more acceptable as they remove heavy metals from soil and beautify the contaminated environment. They give economic benefits (cut flowers and perfume production) to government and local people and the most important reality is that many of them are not edible so they do not enter into the food chain (Prasad, 2016).

Plants selection for phytoremediation is generally done taking into consideration producing a high amount of biomass, fast growth, they should have the capacity to accumulate high concentration of heavy metals in their shoot and be healthy to contain a large amount of soft tissues. In plants deposition of heavy metals occur in the cell wall, vacuole, and metabolically inactive tissues. Once inside the plant, chemicals can be stored in the stem, leaves or roots, they may change into less harmful chemicals within the plant or changed into gases that are released into the air as the plant transpires (Lu *et al*., 2014).

Phytoremediation is a very useful, eco-friendly safe, and cheap way to clean up contaminants. In the present study, the focus is to plan and find out feasible *in-situ* and *ex-situ* techniques of phytoremediation by using ornamental plants for the remediation of polluted water and soil released and present in the Amanishah Nala (Dravyavati River). Dravyavati river runs throughout the Jaipur city (47.5km) starting from the hills of Amer (northeast) to the southwest of the city and at last, it merges with Dhund River. Amanishah nala is divided into 10 sub-zones (Randhawa and Chandra, 2017). Sites selected for phytoremediation are the Sitapura Industrial area and the Sanganer Industrial area of Jaipur city. Jaipur city the capital of Rajasthan which is undergoing urbanization and industrialization and steep increase in population is around 39,09,333 (world Urbanization prospects, census 2011).

The largest drainage system of Jaipur city is Dravyavati river in which all the waste from industries, houses and municipalities are discharged (Sharma and Pandey, 2017). Seepage of this water pollutes the underground water which is used in drinking. Farmer uses this water directly in growing vegetables and other crops and by this, the heavy metals enter into the food chain ultimately causing health problems. There are many physiological processes in plants for phytoremediation like Phytoextraction, Phytostabilization, Phytodegradation, Phytostimulation, Phytovolatilization, Rhizofiltration, and Phytodesalination.



Type of Phytoremedial activities performed by plants: (source:www.researchgate.net)

Types of phytoremediation Mechanism

Type	Process involved	Pollutants treated	Examples
Phytoextraction	Plants are used to concentrate and accumulate of contaminants in harvestable plant tissues (leaves and shoot)	Cadmium (Cd), cobalt (Co), nickel (Ni), mercury (Hg), lead (Pb), selenium (Se), zinc (Zn)	*Rumex crispus, Viola baoshanensis* (Zhuang *et al*., 2007)
Rhizofilteration	Absorption, precipitation and concentration of heavy metals by Plant roots.	Cadmium (Cd), cobalt (Co), chromium (Cr), nickel (Ni), mercury (Hg), lead (Pb), phenolic compounds, radioactive isotopes	*Brassica juncea, Helianthus annus* (Dushenkov *et al*.,1995)
Phytostimulation	The stimulation of microbial and fungal degradation by release of exudates in to the root zone (rhizosphere)	Petroleum hydrocarbons, Polyaromatics, Benzene, Toluene, Atrazine	*Pisum sativum* (Paw *et al*., 2019)
Phytodegradation	The degradation of complex organic molecules into simple molecules and incorporation of these molecules into plant tissues	Textile dyes, petroleum hydrocarbons, chlorinated solvents, herbicides	*Gaillardia grandiflora* (Chandanshive *et al*,2018), *Impatiens balsamina* (Cai *et al*., 2010)
Phytostabilisation	Involving absorption and precipitation of heavy metals by plants, prevent their migration to ground water or air	Inorganic compounds: Arsenic, Cadmium, Copper, Chromium, Zinc, Mercury etc.	*Atriplex lantiformis* (Mendez and Mair, 2008)
Phytovolatizatio	The contaminants can be removed from the plant by transpiration or evaporation in volatile form.	Mercury (Hg), selenium (Se), arsenic (As), chlorinated compound	Willow, alfa-alfa (Limmer and Burken,2016) Poplars (Gordon *et al*., 1998)
Phytodesalination	Decrease in salinity and sodium concentration	Salt, sodium	*Tecticornia indica* (Zorring *et al*., 2012)

Their are more than 18,000 species of flowering plants found in India which is more than 7% of total plant species found in India (Hedge *et al.*, 2013).

In Rajasthan, almost 480 species of plants are reported in which approximate 107 are of trees, 55 of shrubs, 215 of herbs, 45 of climbers, 40 of grasses, and 15 of lower plants (www.rajasthantrekking.com).

Many flowering plants have been reported for phytoremediation capability towards heavy metals absorbtion from contaminated soil viz. *Hibiscus, Helianthus, Duranta, Nerium, Jasmine, Combratum, Catharanthus, Rose, Tabernaemontana, Dahlia, Chrysanthemum, Tagetes,* etc.

In the present research proposal the experiment will be framed for the screening and phytoremediation (*In-situ* and *Ex- situ*) qualities of three ornamental plants viz. *Dianthus barbatus* (Sweet William), *Cosmos bipnnatus* (Mexican aster) and *Gaillardia aristata* (Blanket flower) are selected to check out their capability towards remediation and removal of accumulated heavy metals and beautify the wasteland and contaminated sites of Dravyawati River (Amanishah Nala).

***Dianthus barbatus* (Sweet William)**

Fig- *Dianthus barbatus* (Source: ast.wikipedia.org)

Center of origin- Southern Europe and parts of Asia

Botanical characters: *Dianthus barbatus* (sweet William) is a species of flowering plants of family Caryophyllaceae. The family is commonly called 'Pink' or 'chickweed' family. It has become a popular ornamental garden plant. It is an herbaceous biennial or short-lived perennial plant growing to 13–92 cm tall, Inflorescence is dichasial cyme with flowers in a dense cluster at the top of the stems. Flowers are pedicellate, actinomorphic, bisexual, hypogynous, and pentamerous. Leaves are simple and stipulate. *Dianthus* start to bloom from late spring to early summer.

Economic importance: *Dianthus barbatus* is used as ornamental flowering plants which is generally used in boarders, pots and as a cut flower (Gupta *et al.*, 2004). It is used in the treatment of urinary difficulties in China, the entire plant is used as a bitter tonic herb that stimulates the urinary system and digestive system. Flowers of this plant attracts bees, birds, and butterflies.

Classification (according to Bentham and Hooker)

Kingdom- Plantae
Division- Angiospermae
Sub-division- Dicotyledonae
Class- Polypetalae
Series- Thalamiflorae
Order- Caryophyllales
Family- Caryophyllaceae
Genus- *Dianthus*
Species- *barbatus*

2. *Cosmos bipnnatus* (Mexican aster)

Fig: *Cosmos bipinnatus* (source: pintrest.com)

Center of origin- Mexico

Botanical characters: *Cosmos bipinnatus* a herbaceous plant grown in full sun as an annual beautiful flowering plant with a height of 3 to 6 feet. Leaves are simple, opposite, pinnately cut in too deep lobes appearing compound. The inflorescence is capitula with ray and disc florets. *Cosmos* blooms in summer and rainy season with large showy flowers occur in many colors like pink, red, white, violet, and lavender which attracts butterflies, bees and birds (Jang *et al.*, 2008).

Economic importance: *Cosmos bipinnatus* used as ornamental flowering plants. It is used in the treatment of intermittent fever, jaundice. In South Africa, it is used to cure headache and stomach disease by the Basotho tribe while the other ethnic group Afrikaans use *C. bipinnatus* to control head mice and bad bugs.

Classification (according to Bentham and Hooker)

Kingdom- Plantae
Division- Angiospermae
Subdivision- Dicotyledonae
Class- Gamopetalae
Series- Inferae
Order- Asterales
Family- Asteraceae
Genus- *Cosmos*
Species- *bipinnatus*

3. ***Gaillardia aristata*** **(Blanket flower)**

Fig- *Gaillardia aristata* (source: gardensoul.com)

Center of origin- North, and south America

Botanical characters: *Gaillardia aristata* is an annual or perennial herb that blooms throughout the year and requires very low maintenance, attaining height around 80 centimeters. The leaves are basal or alternately arranged and glandular in most species. The inflorescence is a solitary flower head that has many rays and disc florets.

Economic importance: Blanket flower is a native perennial wildflower useful for adding species diversity in native plant seed mixes for the rehabilitation of disturbed sites. Blanket flower is suitable

for use as an ornamental wildflower in low maintenance or naturalistic landscapes. It has utility as a cover and food source for pollinators, wildlife, and lives.

Classification (according to Bentham and Hooker)

Kingdom- Plantae

Division- Angiospermae

Subdivision- Dicotyledonae

Class- Gamopetalae

Series- Inferae

Order- Asterales

Family- Asteraceae

Genus- *Gaillardia*

Species- *aristata*

REVIEW OF LITERATURE

Azeez *et al.,* (2020) reported that the addition of Urea and EDTA enhanced the metal uptake in the plants by more than 100% and some of the plant species like *Corchorus olitorius* and *Hibiscus cannabinus* could not tolerate the metals.

Mushtaq *et al.,* (2020) reported that microbial aided phytoremediation by using *Catharanthus roseus* could be a potential method for removal of cadmium (Cd), chromium (Cr), and copper (Cu), while the addition of organic compounds in presence of heavy metal increases the growth of a plant.

Shrestha *et al.,* (2019) reported that phytoremediation can be enhanced in heavy metal contaminated soil by improving soil properties, plant productivity, reducing heavy metal solubility and uses of organic amendments.

Nanda and Pradhan, (2019) studied that heavy metals are extracted by ornamental plants as well as beautification of dirty areas. *Phoenix dactylifera, Olea emopaea, Pongamia pinnata, Cassia samea, Helianthus annum, Amaranthus caudatus, Mirabilis jalapa,* and *Tagetes erecta* have used for contaminated

environment and results were very much positive.

Liu *et al*., (2018) studied the growth responses, cadmium (Cd) accumulation and uptake capability of six popular Compositae species, namely, *Taraxacum mongolicum, Tagetes erecta* L., *Tagetes patula* L., *Zinnia elegans Jacq., Centaurea cyanus* L. and *Gerbera jamesonii Bolus* under Cd stress were investigated. According to their investigation, it was shown that *Tagetes erecta* L. and *Tagetes patula* L. had strong tolerance and accumulation capability to cadmium (Cd), therefore they can become potential hyperaccumulators in phytoremediation of Cd-contaminated soils.

Utomo and Mangkoedihardjo (2018) studied the ability *Helianthus annuus, Zinnia elegans,* and *Impatiens balsamina* to remove chromium (Cr) from the soil is enhanced by microbial activity on the root zone.

Sharma *et al*., (2018) reviewed that techniques used for remediation of heavy metal contaminated soil are chemical, physical and biological. These techniques have their own setback so if we use combination of these techniques, it will be more effective.

Alaboudi *et al*., (2018) studied that the value of translocation factor (TF) for cadmium (Cd) increased when the concentration of cadmium (Cd) in soil increases and the value of translocation factor (TF) is decreased with increasing the concentration of lead (Pb), *Helianthus annuus* is more approving for cadmium (Cd) uptake compared to lead (Pb).

Pandey *et al*., (2015) proved by their experiments that *Lantana camara* and *Ipomoea carnea* showed good translocation of heavy metals from root to shoot but *Solanum surattense* accumulate most of the metal in roots it can be concluded that *Lantana camara* and *Ipomoea carnea* can be used as phytoextractor and *Solanum surattense* as phytostabilizer.

Mani *et al*., (2015) studied that phytoextraction is the process of using plants, especially hyperaccumulator plants, to extract or concentrate metals or organics to harvestable biomass, whereas phytodegradation is the process of using the root exudates of plants to stimulate microbial activity whereby the enhancement of mineralization at the root-soil interface is attributed to microorganisms associated with the root surface.

Wu *et al*., (2015) studied that phytoremediation is a cost-effective green technology based on the use of plants to remove, metabolize, assimilate, or adsorb hazardous materials from the soil.

Swapna *et al.,* (2014) studied the accumulation pattern of heavy metals aluminium (Al), cadmium (Cd), iron (Fe), mercury (Hg), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) in *Chromolaena odorata* and observed significant variations in the quantity of accumulation as well as distribution among plant parts like root, stem, and leaf. The maximum quantity of each metal was accumulated in the root as compared to leaves and stem.

Khan and Gaikwad (2013) investigated that atrazine (herbicide) was reduced at a faster rate in soil when *Brassica juncea* L. was planted than the unplanted soil.

Shivhare and Sharma (2012) investigated that the overall length of the plant decreases as the concentration of nickel (Ni) and lead (Pb) is increased in the soil.

Karami and Samsuddin (2010) studied many enhancer methods and applied parallel with phytoremediation like chelating agents, microorganisms (arbuscular mycorrhizal fungi, plant growth-promoting rhizobacteria (PGPR), lowering the pH and increasing the electrode potential (Eh) and concluded that multi approaches enhanced the removal rate of contaminants rather than a single technique.

Abdolkarim and malayeri (2007) studied the native accumulator plants, *Euphorbia cheiradenia, Scariola orientale, Centurea Virgata, Gundelia tounefortii,* and *Eleagnum angustifolia* could accumulate heavy metals in different organs among them *E.cheiradenia* recommended as best phytoaccumulator.

Zhuang *et al.,* (2007) studied the phytoremediation ability of six plants (*Vertveria zizanioides, Dianthus chinensis, Rumex k-1(R. upatientia x R. timschmicus), R. crispus, R. acetosa, Viola baoshanensis* and *sedum alfredi* in which *Rumex crispus* is most appropriate species for heavy metals extraction.

Phytoremediation is in-situ, non-intrusive, aesthetic pleasing, ecologically benign, effective, and socially accepted technology to remediate polluted soils (Alkortal *et al.*,2001; Garbisu *et al.*,2002).

STATEMENT OF THE RESEARCH PROBLEM

Phytoremediation is the use of living green plants for *in situ* removal of contaminants from contaminated soil, water, sediments, and air.

"Estimation of Phytoremedial capability of some Ornamental Plants against water and soil pollution in selected sites of Dravyavati river, Jaipur, Rajasthan".

OBJECTIVES OF THE STUDY

- To perform Physicochemical analysis of samples collected from various sites of the Dravyavati river.
- To do selection of ornamental plants grown in different seasons (winter, rainy and summer) of Jaipur.
- To set *In-situ* and *Ex-situ* experimentation for the comparative study of phytoremediation potential against heavy metals.
- To evaluate Physicochemical, qualitative and quantitative parameters of plants against heavy metal remediation.
- To screen out and suggest the most potential ornamental plant and technology for phytoremediation activity at selected sites.

SCOPE OF THE STUDY

Due to rapid industrialization, anthropogenic activities and pollution, many pollutants and noxious metals have accelerated dramatically in the soil and water causing bioaccumulation and biomagnification in the food chain and causes non curable health problems in living organisms. The screening of ornamental plants as potential phytoremediator for metals can be a new alternative approach for polluted sites rehabilation of pollution effected water and soil which works for the beautification of the sites and surroundings. The farmer in close vicinity can grow these ornamental plants which can solve dual purpose i.e. Income generation and beautification. Mixed cropping with other conventional crops can also be suggested for future.

SIGNIFICANCE OF THE STUDY

Phytoremediation of industrial effluent contaminants by ornamental plants is more effective because they clean up the contaminated wasteland by beautification and can boost the economy of marginal farmers having their land in the close vicinity of the polluted sites. Most of the ornamental plants are non- edible therefore the threat of biomagnifications of heavy metals into the food chain is not expected. If this study will be successful then it can be used to reclaim the unused wastelands and polluted sites by beautifying them and generate economic benefits.

LIMITATIONS

- The total flow of Amanishah nala is 47.5 km long but the study will be restricted only to the selected sites where wastewater effluents and soil of the Dravyavati river is highly effected with the pollutants.
- Phytoremediation is a time-consuming process that takes many growing seasons to clean up a site.
- There are more than 18,000 species of flowering plants found in India and this proposal is confined with only three ornamental plants species.

METHODOLOGY

1. Physicochemical analysis of soil and water sample:

The water and soil sample will be collected from the selected sites of Dravyavati river for the physicochemical quantification and experiments set up. The collected samples will be packed in polythene bags and plastic bottles and bring to the laboratory for physicochemical analysis and quantitative detection of heavy metals by Atomic Absorption Spectrophotometer (AAS).

2. Soil characterization and soil preparation for pot experiment:

Samples of the soil (depth 01- 15 cm) will be taken and sieved through a (1cm hole size) sieve. Soil characterization will be done by air-drying under the shade, remove stones, wood particles by hand than crushing of air dried samples by a hammer, sieving of crushed soil samples (mesh size 2mm), and mixing thoroughly to make the samples homogeneous for experiments.

Representative samples will be taken for the characterization of basic properties. Soil properties will be determined by different methods; soil texture by textural triangle, particle size distribution by hydrometer method, soil bulk density by core method (Culley, 1993), electronic conductivity by EC meter, soil pH by pH meter, soil organic matter by Walkley-Black method (Nelson and Sommers, 1982) and cation exchange capacity by ammonium acetate method (Hendershot *et al*., 2006). In soil samples, heavy metals will be determined before and after phytoremediation by atomic absorption spectrophotometer (AAS) and concentration of heavy metals will be compared with normal soil.

3.Selection of plants and experiment setup:

Phytoremediation study will be conducted by using ornamental plants grown in the field (*In-situ*) and pots (*Ex-situ*). In the present study, winter, rainy and summer season flowering plants selected for the experimentation are *Dianthus barbatus, Cosmos bipinnatus,* and *Gaillardia aristata*.

Earthen pots (7-8 inch) will be prepared by using, mixing and making different combination of polluted soil sample collected from the sites. These pots will be lined with polythene bags to avoid leaching. Different experimental setup with a varied combination of water and soil samples along with some organic waste materials will be prepared for all three flowering plants selected.

Different combination mixture of waste (living and nonliving material like Gravel, Organic manure, Clay, locally available husk) along with the polluted soil samples collected from selected sites will be made in a triplicate along with standard control for each combination as well as for all three flowering plants selected for research. Seeds of each plant species will be sown in each pot to obtained 5 plants of each species and irrigated by wastewater effluents. All the ornamental plants will be uprooted at a time interval of 15, 30, 45, 60, 75 days. The soil will also be removed from all the pots after the experiment for analysis.

4. Screening of Ornamental Plants for Phytoremediation Qualities:

The fresh weight will be measured after washing the plant firstly with tap water and then distilled water to remove soil and other dirt particles from the plant surface. The plants will be first dried in air and then oven at 120°C overnight to remove all the moisture and dry weight will be measured by using the balance. The moisture content in the plants will be calculated after measuring the fresh and dry weight of the plants. The quantitative concentration absorption of heavy metals in plants and soil samples will be measured by atomic absorption spectrophotometer (AAS).

AAS (Atomic absorption spectrophotometer) – AAS is very reliable and common technique for detecting metals and metalloids in samples. The principle behind AAS is that free atoms (gas) can absorb radiation at specific frequency.Eliments to be analysed need to be in atomic state, atomization (breaking of molecules in

to atoms) is done by exposing analyte to very high temprature.

We will calculate the translocation factor (TF) and bioconcentration factor (BCF) for evaluation of plants for phytoremedial capability.
Translocation factor (TF) is the ratio of the amount of heavy metals translocated by the plant from roots to its different parts (stem, leaves and flowers).
Bioconcentration factor (BCF) is the ratio of the concentration of the substance in an organism to the exposure condition.

TF=Conc. of heavy metal in shoot/Conc. of heavy metal in root

BCF= Conc. of heavy metal in plants/ Conc. of heavy metal in soil

Bioconcentration factor (BCF) and translocation factor (TF) are essential for the selection and evaluation of plants for phytoremediation purposes (Wu *et al*., 2010, Ehsan *et al*., 2016).
A plant can be used in phytoremediation if the TF (translocation factor) and BCF (bioconcentration factor) measured (>1) more than one (Yoon *et al*., 2006).

5. Screening of best phytoremediation plants:

The concentration of heavy metals will be analyzed by AAS to determine the amount of heavy metals in plants. Results with most effective accumulator plant will be selected for removing of soil heavy metals in experimental phytoremediation.

MONTH WISE PLAN OF WORK

1-24 months	Review of literature throughout the study
1-3 months	Soil and water sample Collection from selected sites
3-6 months	Analysis of soil and water samples
6-12 months	Experiment setup and Biochemical and histochemical studies in selected plants
12-20 months	Screening of ornamental plant as best phytoremediation plant
20-24 months	Thesis preparation

Total Time (expected) = 24 Months

WORK PLAN (Month wise) GRAPHICAL REPRESENTATION

Activity/Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Review of Literature																								
Sample Collection																								
Soil and Water Analysis																								
Study of Morphological Characters																								
Analysis and interpretation of results																								
Thesis preparation																								

REFERENCES

1. Abdolkarim Rad C. and Malayeri B., "Removal of heavy metals by Native Accumulator plants", International Journal of Agriculture & Biology, **9** (3), 462-465, (2007).
2. Alaboudi K.A., Ahmed B., and Brodie G., "Phytoremediation of Pd and Cd Contaminated Soil by Using Sunflower (Helianthus annuus) plant", Annals of Agriculture Sciences, 63, 123- 127, (2018). DOI: 10.1016/j.aoas.2018.05.007
3. Alkortal., and Garbisu C, "Phytoremediation of Organic Contaminants", Bioresour Technol., **79**(3), 273-276, (2001).
4. Azeez J.O, Olowoboko T.B., BadaB.S. and Odedina J.N., "Evaluation of soil metal sorption characteristics and heavy metal extractive ability of indigenous plant species in Abeokuta, Nigeria", International Journal of phytoremediation, **22** (8), 872-884, (2020). DOI; 10.1080 /15226514 .2020. 1717433
5. Blaylock M. J., Huang J. W., "Phytoextraction of metals", In: Raskin I., Ensley B. (eds) Phytoremediation of toxic metals: Using plants to clean up the environment, Wiley-Interscince, New jersey, 53-88, (2000).
6. Cai Z., Zhou Q. X., Peng S. W. and Li K.N., "Promoted biodegradation and microbiological effects of petroleum hydrocarbons by *Impatiens balsamina* with strong endurance", Journal of Hazardous Material, **183** (1-3), 731-737, (2010).
7. Cempel M., and Nikel G., "Nickel: a review of its sources and environmental toxicology", Pol. J. Environ. Stud. **15** (3), 375-382, (2006).
8. Chaney R.L., Brown S.L. and Li Y.M., "Progress in Risk Assessment for Soil Metals, and In-situ Remediation and Phytoextraction of metals from Hazardous contaminated soil", Phytoremediation: State of Science, 2000 May 1-2. Botson, MA.232-240, (2000).
9. Chhotu D. J. and Fulekar M.H., "Phytoremediation of heavy metals: Recent techniques", African Journal of Biotechnology vol. **8**(6), pp. 921-928, 20 March, (2009).
10. Culley J.L.B., in ed.: Carter M.R., Density and Compressibiliity, In: Soil Sampling and Methods of Analysis, pp. 529-540 (1993).
11. Cunningham S.D., Ow D.W. "Promises and prospects of Phytoremediation", Plant Physiol, **110** (5), 715-719. (1996).
12. Dushenkov V., Kumar P. B. A. N., Motto H. and Raskin I., "Rhizofiltration: The

Use of Plants to Remove Heavy Metals from Aqueous Streams", Environmental Science and Technology, **29** (5), 1239-45, (1995).

13. Ehsan N., Ahmad S. and Arshad M., "Remediation of Heavy metal-contaminated Soil by Ornamental Plant Zinnia (*Zinnia elegance L.*)", Asian Journal of Chemistry, **28** (6), 1338-1342, (2016).
14. Garbisu C., Hernandez-Allica J., Barrutia O., Alkorta I., and Becerril J.M., "Phytoremediation: a technology using green plants to remove contaminants from polluted areas", Rev Environ Health, **17** (3), 173-188, (2002).
15. Gorden M., Choe N., Duffy J., Ekuan G., Heilman P., Muiznick I., Ruszaj M., Shurtleff B. B., Strand S., Wilmoth J. and Newman L.A., "Phytoremediation of Trichloroethylene with hybrid Poplars", Environmental Health Perspectives, **106** (Suppl 4), 1001-1004, (1998), DOI: 10.1289/ehp.98106s41001.
16. Gupta Y.C., Rana N., Dhiman S. R., and Jain R., "Floral biology and seed setting studies in *Dianthus barbatus* L. **7** (3-4), 11-17, (2004).
17. Hendershot W.H., Lalande H. and Duquette M., in ed.: M.R. Carter, Ion Exchange, and Exchangeable Cations, In Soil Sampling and of Analysis, Lewis Publishers, Boca Raton, FL, pp. 197-206 (2006).
18. Hedge M., Suresh K., Sashidharan K.R., Singh B. G., Raghunath T. P. and Krishnakumar N., "Indian plant species", Institute of Forest Genetics and Tree Breeding, Indian Council of Forestry Research and Education, Coimbatore 641002.
19. Jang I.C., Park J.H., Park E., Park H.R., and Lee S.C., "Antioxidative and Antigenotoxic Activity of Extracts from Cosmos (*Cosmos bipinnatus*) Flowers", Plant Foods for Human Nutrition, **63**, 205-210, (2008), DOI 10.1007/s11130-008-0086-8.
20. Karami A. and Shamsuddin Z. H., "Phytoremediation of heavy metals with several efficiency enhancer methods", African Journal of Biotechnology, **9** (25), 3689-3698 (2010).
21. Khan S. and Gaikwad R. S., "Phytoremediation potential of *Brassica juncea* L. with reference to atrazine", International Res. Journal of Science and Engineering, (1), 5-9, (2013).
22. Kumar P.B.A., Dushenkov V, Motto H., and Raskin I, "Phytoextraction: the use of plants to remove heavy metals from the soil", Environ Sci Technol, **29** (5), 1232-1238, (1995).
23. Liu Z., Chen W., and He X., "Evaluation of hyperaccumulation potentials to cadmium (Cd) in six ornamental species (Compositae)", International Journal of

Phytoremediation, **20** (14), 1464-1469, (2018).

24. Lu H., Li Z., Fu S., Amende Z., Gasco G., and Paz-Ferreiro J., "Can biochar and Phytoextractors be jointly used for Cadmium remediation?", PloS One, **9** (4), https://doi.org/10.1371/journal.pone.0095218 , (2014).
25. Mani D., Kumar C., Patel N.K. and Sivakumar D., "Enhanced clean-up of lead-contaminated alluvial soil through *Chrysanthemum indicum* L.", International journal of Environmental science and technology, **12** (4), 1211-1222 (2015), doi: 10.1007/s13762-013-0488-5.
26. McGrath S.P., Chaudri A.M., and Giller K.E., "Long-term effects of metals in sewage sludge on soil", microorganism and plants, J Ind Microbiol, **14** (2), 94-104, (1995).
27. Mendez M.O. and Maier R.M., "Phytostabilisation of mine tailings in arid and semiarid environments- An emerging remediation technology", Environmental Health Perspective. **116** (3), 278-283 (2008)
28. Mushtaq M.U., Iqbal A., Nawaz I., and Mirza C.R., "Enhanced uptake of Cd, Cr, and Cu in *Catharanthus roseus (L.)* G. Don by Bacillus cereus: application of moss and compost to reduce metal availability", Environment Science and Pollution Research, **33,** DOI: 10.1007/S11356-020-08839-5, (2020).
29. Nanda S.P. and Pradhan A., "Role of Ornamental Plants in Phytoremediation of Environmental Pollutant and Economic Benefits", GTCE-41(2019).
30. Nabanpote W., Meesungnoen O. and Prasad M.N.V., *Potential of Ornamental Plants for Phytoremediation of Heavy Metals and Income Generation,* M.N.V. Prasad, Bioremediation and Bioeconomy, ELSEVIER, ISBN:978-0-12-802830-8 (2016).
31. Nelson D.W. and Sommers L.E., in eds.: A.L. Page, R.H. Miller and D.R. Keeney, "Total Carbon, Organic Carbon, and Organic Matter, In: Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties", Madison, Wisconsin: SSSA, pp. 539-579 (1982).
32. Pandey S., Battacharya T., and Chakraborty S., "Metal Phytoremediation Potential of Naturally Growing plants on Fly Ash Dumpsite on Patratu Thermal Power Station, Jharkhand, India", International Journal of Phytoremediation, **18** (1), 87-93, (2016), DOI;10.1080/15226514.2015.1064353.
33. Patil A. and Gawande S., "Phytoremediation: Green Technology: A review", International Journal of Engineering Science and Computing, **8** (5), 17496-17499, (2018).
34. Paw M. H., Ratomski P. and Golimowski W., "Pea cultivar Blauwschokker for the

phytostimulation of biodiesel degradation in agricultural soil", Environmental Science and pollution Research, **26** (11), 34594-34602, (2019), DOI: 10.1007/s11356-019-06347-9.

35. Prasad M.N.V. and Freitas H.M.O., "Metal hyperaccumulation in plants -Biodiversity prospecting for phytoremediation technology", Electronic Journal of Biotechnology, ISSN: 0717-3458 (2003).
36. Randhawa A. and Chandra T., "Revitalization of Dravayawati River, Jaipur, India: A waterfront development project", Journal of Geography and Regional Planning, **10** (3), 28-38, (2017), https://doi.org/10.5897/JGRP2017.0620.
37. Reeves R.D., Baker A.J.M., Borhidi A., Berazain R., "Nickel hyperaccumulating plants from the ancient serpentine flora of Cuba", New Phytol, **133** (2), 217-224 (1996).
38. Sarwar N., Malhi S.S., Zia M.H., Naeem A., Bibi S. and Farid G., "Role of mineral nutrition in minimizing cadmium accumulation by plants. J. Sci. Food Agric., **90** (6), 925-937, (2010), Doi: 10.1002/jsfa.3916.
39. Saxena P.K., Krishnaraj S., and Dan T., "Phytoremediation of heavy metal contaminated and Polluted Soils", In Prasad M.N.V., Hagemeyer J, editors. Heavy Metal Stress in Plants: from Molecules to Ecosystem. Berlin: Springer, 305-329, (1999).
40. Sharma and Pandey., "Phytoremediation of Heavy Metal Lead by Fodder Grass Lasiurus scindicus in polluted soil and water of Dravyawati River", American Journal of environmental sciences, **13** (2), 167-171, (2017).
41. Sharma S., Tiwari S., Hasan A., Saxena V. and Pandey L.M., "Recent advances in conventional and contemporary methods for remediation of heavy metal-contaminated soils", 3 Biotech, part of Springer Nature, **8** (4), 216, (2018). http://doi.org/10.1007/s13205-018-1237-8
42. Shivhare L. and Sharma S., "Effect of toxic Heavy metal Contaminated soil on an Ornamental plant *Georgia wild* (Dahlia)", Journal of Environmental & Analytical Toxicology, **2** (7), 1-3, DOI:10.4172 / 2161 – 0525. 1000156. (2012).
43. Smith B., "Remediation update funding and remedy", Waste Manage. environ., **4**, 24-30 (1993).
44. Shrestha P., Belliturk K. and Gorres J. H., "Phytoremediation of Heavy metal-contaminated Soil by Switchgrass: A Comparative Study Utilizing Different Composts and Coir fiber on Polluted Remediation, Plant Productivity and Nutrient Leaching", International Journal of Environment Research and Public Health, **16** (7), (2019), DOI: 10.3390/ijeph16071261.

45. Soni S. and Jain S., "A review on phytoremediation of heavy metals from soil by using plants to remove pollutants from the environment", International Journal of Advanced Research, ISSN 2320-5407, **2** (8), 197-203, (2014).
46. Stanley I.R.O., Grace O.I., Ebere P.A., Abraham O.A., and Ibe K.E., "Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review", Journal of Toxicology, http://doi.org/10.1155/2018/2568038.
47. Swapna K. S., Shackira A.M., Abdussalam A. K. and Nabeesa S., "Accumulation pattern of Heavy Metals in *Chromolaena odorata* (L.) King & Robins. Grown in nutrient solution and soil", Journal of Stress Physiology & Biochemistry, **10** (2), 297-314, (2014).
48. Tangahu B.V., Abdullah S.R.S., Basri H., Anuar N., and Mukhlisin M., "A Review on Heavy Metals (As, Pd, and Hg) uptake by plants through phytoremediation", International Journal of Chemical Engineering, https://doi.org/10.1155/2011/939161, (2011).
49. Utomo A.A. and Mungkoedihardjo S., "Preliminary Assessment of mixed plants for Phytoremediation of Chromium Contaminated soil", Current world Environment, ISSN; 0973-4929, **13** (special issue 1), 22-24 (2018), DOI: 10.12944/CWE.13.Special-Issue1.04
50. Wu G., Kang H., Zhang X., Shao H., Chu L. and Ruan C., "A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities", Journal of Hazard mater, **174** (1-3), 1-8, (2010), doi: 10.1016/j.jhazmat.2009.09.113
51. Wu Z., Banuelos G. S., Lin Z. Q., Liu Y., Yuan L., Yin X. and Li M., "Biofortification and phytoremediation of selenium in China", Front. Plant Sci., **6** (6), 136, (2015), doi:10.3389/fpls.2015.00136.
52. Zhuang P., Yang Q. W., Wang H.B. and Shu W.S., "Phytoextraction of heavy metals by eight plant species in the field", Water Air and Soil Pollution, **184** (1), 235-242, (2007) , doi:10.1007/s11270-007-9412-2.
53. Zorring W., Rabhi M., Ferchichi S. and Smaoui A., "Phytodesalination: a solution for salt-affected soils in arid and semi-arid regions", Journal of Arid Land, **22** (1), 299-302, (2012).
54. www.hyperaccumulators.org, (2015).
55. www.rajasthantrekking.com

56. world Urbanization prospects, census (2011).
57. (ast.wikipedia.org) https://encrypted-tbn0.gstatic.com/images?q=tbn%3AANd9GcQFq1lNapnTzvD_ns_sZxUIKgZNH9Y3gW1y6A&usqp=CAU
58. (gardensoul.com) https://gardeningsoul.com/wp-content/uploads/2020/03/Add-Gaillardia-to-the-Xyriscaped-Garden.jpg
59. (Pinterest.com) https://nl.pinterest.com/pin/96757091983099056/
60. (www.researchgate.net) https://www.researchgate.net/figure/Schematic-diagram-of-different-approaches-of-phytoremediation_fig3_268148634